Skeletal Response to Simulated Weightlessness: A Comparison of Suspension Techniques

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The skeletal response to simulated weightlessness was studied in rats subjected to two different methods of suspension. Skeletal unloading of the hind limbs for a two week period was achieved by use of either a back harness or tail traction. In comparison to pair-fed control rats, back-suspended rats failed to gain weight whereas tail-suspended rats exhibited normal weight gain. Quantitative bone histomorphometry revealed marked skeletal abnormalities in the proximal tibial metaphysis of back-suspended rats. Loss of trabecular bone mass in these animals was due to a combination of depressed longitudinal bone growth, decreased bone formation, and increased bone resorption. In contrast, the proximal tibia of tail-suspended rats was relatively normal by these histologic criteria. However, a significant reduction in trabecular bone volume occurred during 2 weeks of tail suspension, possibly due to a transient inhibition of bone formation during the early stages of skeletal unloading. Lack of weight gain in back-suspended rats may be indicative of a pronounced stress response during which corticosteroids adversely affected the skeleton. Maintenance of normal weight gain by tail-suspended rats provides evidence for the less traumatic nature of this method of suspension. Our findings indicate that tail suspension may be a more appropriate model for evaluating the effects of simulated weightlessness on skeletal homeostasis.

LOSS OF SKELETAL mass is a potentially serious consequence of long-term space flight. Skylab astronauts exhibited a significant decline in the bone mineral density of the calcaneus after 84 d of orbital flight (17). Rats placed in orbit aboard Soviet Cosmos biosatellites were characterized by a reduced mass of trabecular bone (8,23) and an accumulation of marrow fat (8). Although bone resorption was not altered in these animals (1), weightlessness inhibited periosteal bone formation (14,22) and induced a decline in the metaphyseal osteoblast population (8). These findings suggest that bone loss during space flight is due primarily to diminished bone formation.

The relative infrequency and prohibitive expense of space experimentation emphasize the need to develop ground-base models of weightlessness. Bed rest has been used to mimic space flight in humans. Although bone loss occurs in adults subjected to prolonged bed rest (2), restrictions on the use of invasive techniques (i.e., bone biopsy) in humans make it difficult to determine the pathogenesis of the osteopenic changes. For this reason, animal models of weightlessness are of considerable interest. As an initial approach, total mechanical unloading of the hind limbs was achieved by suspending rats with an orthopedic harness attached to their backs (13). Skeletal alterations in back-suspended rats were determined to be comparable in nature but more severe in extent than the bone changes observed in rats subjected to space flight (21). However, the traumatic nature of back suspension, as evidenced by lack of weight gain (21), is an undesirable aspect that complicates interpretation of the results. Consequently, a less traumatic method of simulating weightlessness was developed in which rats are suspended by their tails. The purpose of this report is to compare the skeletal response of rats subjected to simulated weightlessness by back or tail suspension.

MATERIALS AND METHODS

In a prior study, 42-d-old male Munich Wistar rats that weighed an average of 130 g were suspended

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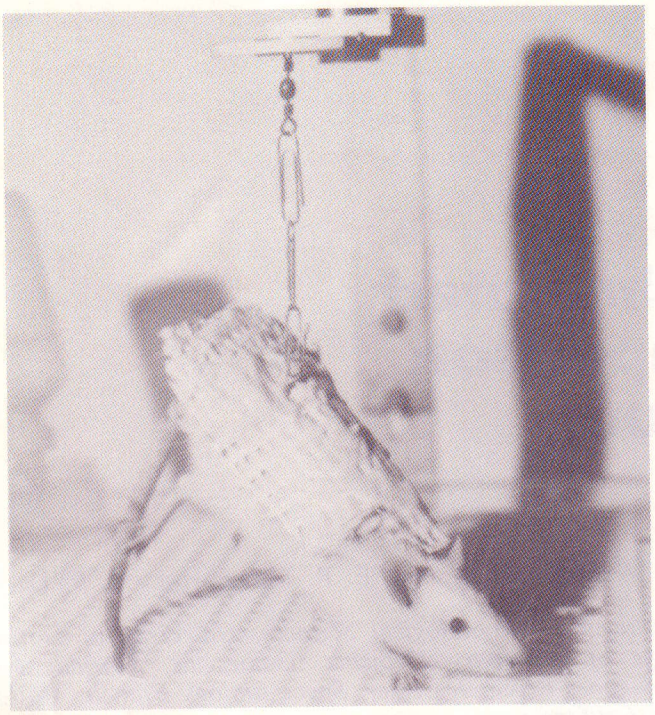

Fig. 1. Rat suspended from an orthopedic harness bonded to its back to induce a cephalad fluid shift and total mechanical unloading of the hind limbs.

from a freely rotating aluminum beam by means of an orthopedic harness bonded to their shaved backs (Fig. 1). The methods have been described previously in detail (13,21). The rats were anesthetized by means of an i.m. injection of pentobarbital (50 $mg \cdot kg^{-1}$ body weight). After attachment of an orthopedic harness, rats were suspended with a headdown tilt (~30°) to initiate a shift of body fluids similar to that experienced during space flight. Total mechanical unloading of the hind limbs occurred, but the forelimbs remained in contact with the floor of the model to allow movement and continuous access to food and water. Ten rats were maintained on this model for a 2-week period during which their weight and food consumption were monitored. Ten control rats were housed individually without attachment of the orthopedic harness to their backs. Their food consumption was matched to that of the suspended rats. All animals were housed in a room maintained at 24°C and illuminated with fluorescent light 12 h daily.

In the current study, male Munich Wistar rats that were 43-d-old and weighed an average of 124g were subjected to tail suspension (Fig. 2). Conscious rats were loosely restrained in a towel while their tails were mildly abraded with gauze soaked in 70% ethanol. Tincture of Benzoin was sprayed on the skin for protection against adhesive tape irritations and allowed to dry. A strip of orthopedic tape, attached to a plastic suspension bar, was applied to the lateral sides of the tail. The tape was then secured by wrapping a strip of stockette around the tail. Each rat was then attached via the plastic suspension bar to a pulley system mounted on the top of an acrylic housing unit. The 10 rats suspended in this manner were allowed freedom of movement and access to food and water. Food consumption and body weight were recorded daily. The control rats were housed individually and pair-fed according to the food consumed by the suspended animals. The room in which the animals were housed was maintained at 24°C with a 12-h light/dark cycle.

To label calcifying tissues (12), all rats were injected intraperitoneally with 10 $mg \cdot kg^{-1}$ body weight of the tetracycline derivative demeclocyline (Lederle Laboratories, Pearl River, N.Y.) 24 h prior to sacrifice. At autopsy, the proximal thirds of the right tibiae were placed in 10% phosphate-buffered formalin for

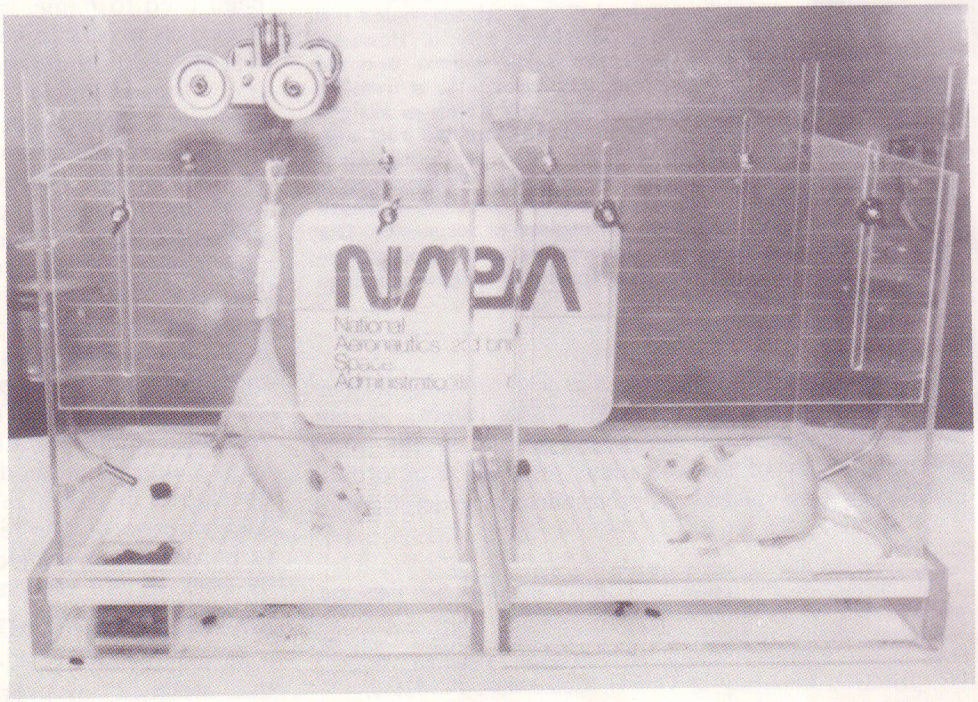

Fig. 2. Skeletal unloading of the hind limbs in a rat subjected to tail suspension. A control rat is seen to the right.

24 h. After dehydration in ethanol, the bone specimens were embedded undecalcified in methyl methacrylate and sectioned longitudinally with an AO/Autocut Jung 1140 microtome. Thin sections (4 μm) were stained according to a modification of Goldner's method (6) for bone histologic analyses. Unstained sections of 10 μm thickness were subjected to an ultraviolet microscopic study of fluorescent tetracycline labels.

To measure the rate of longitudinal bone growth, the distance between the growth plate-metaphyseal junction and the fluorescent tetracycline band that parallels the growth plate was quantified with a calibrated eyepiece micrometer (20) at five equally-spaced sites per section. These measurements were performed under UV illumination in two sections per animal. The rate of longitudinal bone growth was calculated by dividing the distance between the tetracycline band and the growth plate-metaphyseal junction by the time interval between administration of the tetracycline label and sacrifice.

Quantitative bone histomorphometry was performed with the aid of a Merz grid (11) in 4-μm thick sections of the proximal tibial metaphysis. The area sampled was standardized in relation to the growth plate-metaphyseal junction. The number of points superimposed over mineralized tissue (calcified cartilage and bone), bone marrow, and fat were recorded. The fractional area of mineralized tissue, commonly referred to as trabecular bone volume, was determined by dividing the number of points lying over mineralized tissue by the total number of points. The fractional area of fat in the bone marrow was calculated in a similar manner.

The number of osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) adjacent to trabecular bone surfaces of the proximal tibial metaphysis were also quantified. The data are expressed as number of cells per mm trabecular bone perimeter. This latter parameter was determined by recording the number of intersections of the semicircular grid lines with the bone-bone marrow interface. Trabecular bone perimeter was calculated by multiplying the number of intersections by the constant d, which is equal to the distance between grid points (11).

Student's *t*-test was used to evaluate statistical differences between suspended and control groups. P values less than 0.05 are considered to be significant.

RESULTS

Fig. 3 and 4 depict the body weights of suspended and control rats as a function of time. Rats subjected to back suspension failed to gain weight during 2 weeks of simulated weightlessness. These animals weighed significantly less ($p < 0.001$) than pair-fed control rats at the end of the suspension period. In contrast, rats subjected to tail suspension gained weight at a rate comparable to that of the pair-fed control group. At the end of 2 weeks of simulated weightlessness, the weights of tail-suspended and control rats were not significantly different.

The results of quantitative bone histomorphometry in the proximal tibial metaphysis of rats subjected to back and tail suspension for 2 weeks are listed in Table I. A photomicrograph of a representative bone section from which the data were collected is shown in Fig. 5. Back-suspended rats were characterized by a loss of trabecular bone mass, an accumulation of marrow fat, and a depressed rate of longitudinal bone growth. In addition, a decline in the osteoblast population and

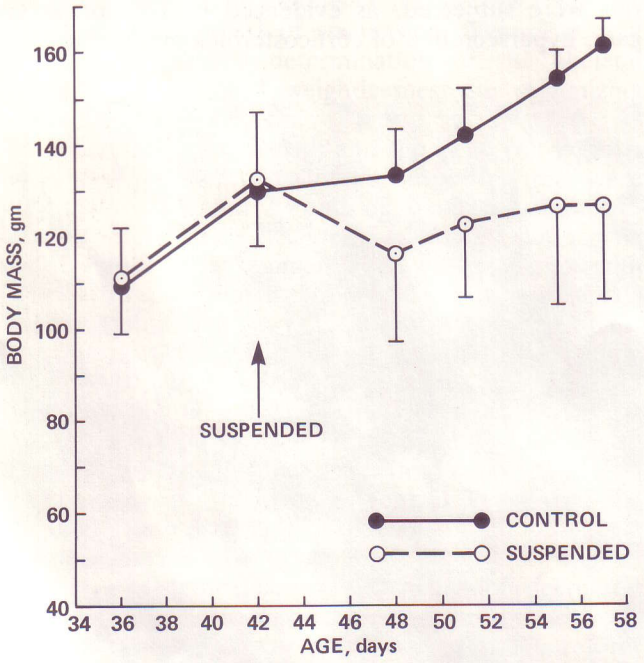

Fig. 3. Body mass as a function of time in back-suspended and pair-fed control rats. Back-suspended rats weighed significantly less ($p<0.001$) than pair-fed control rats at the end of the suspension period.

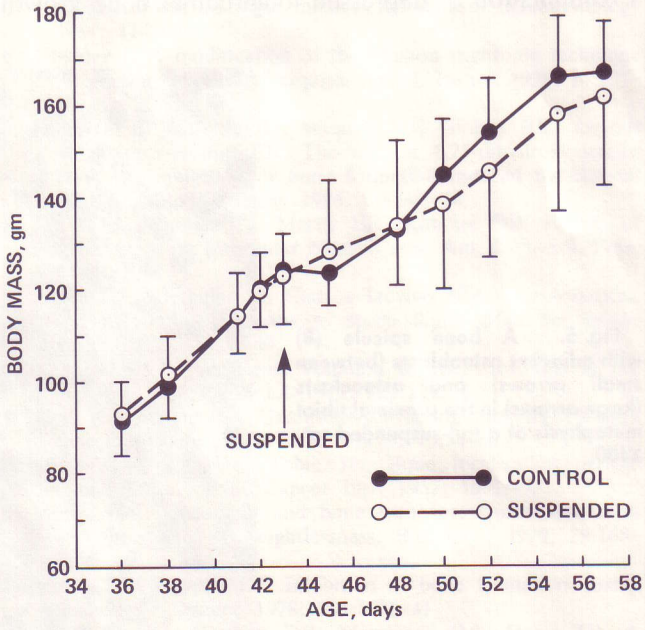

Fig. 4. Body mass as a function of time in tail-suspended and pair-fed control rats. The body masses of tail-suspended and control rats were not significantly different at the end of the suspension period.

TABLE I. QUANTITATIVE BONE HISTOMORPHOMETRY IN THE PROXIMAL TIBIAL METAPHYSIS OF SUSPENDED AND CONTROL RATS.

Method of Suspension	Group	Trabecular Bone Volume (%)	Fat Volume (%)	Longitudinal Bone Growth ($\mu m \cdot d^{-1}$)	Osteo-blasts/mm	Osteo-clasts/mm
Back	Suspended (N = 10)	11.7[a] ± 6.3	18.8[a] ± 10.0	47.1[a] ± 18.9	21.0[a] ± 3.3	4.3[a] ± 1.0
	Control (N = 10)	24.6 ± 7.0	2.8 ± 1.5	125.5 ± 24.6	27.5 ± 2.5	1.7 ± 0.3
Tail	Suspended (N = 10)	17.8[b] ± 2.5	1.1 ± 1.0	142.8 ± 22.9	25.2 ± 5.6	2.2 ± 0.8
	Control (N = 11)	22.0 ± 3.7	0.4 ± 0.5	152.6 ± 15.3	26.4 ± 2.2	2.1 ± 0.4

All values are the mean ± S.D.
[a]$p<0.001$; [b]$p<0.01$

increased numbers of osteoclasts occurred in response to simulated weightlessness. These differences in the skeletal profile of back-suspended and control rats are all highly significant at the level of $p < 0.001$. In contrast to the marked histomorphometric changes detected in back-suspended rats, most bone histologic parameters in the proximal tibiae of tail-suspended rats were not significantly altered. However, tail-suspended rats did exhibit a significant reduction in trabecular bone volume ($p < 0.01$). The decline in trabecular bone volume was at least twofold greater in back-suspended rats (~50% decrease) than in tail-suspended rats (~20% decrease).

DISCUSSION

It was previously determined that suspension of rats for a 2-week period from an orthopedic harness attached to their backs induced marked skeletal abnormalities in the proximal tibial metaphysis (21). Loss of trabecular bone mass in these animals appeared to be due to a combination of depressed longitudinal bone growth, decreased bone formation, and increased bone resorption. In the current study, suspension of rats by the base of their tails for the same length of time did not alter to the same degree skeletal processes in the proximal tibia. Since total mechanical unloading of the hind limbs occurred during both methods of suspension, the data indicate that the skeletal alterations observed in back-suspended rats were not the result of a lack of weightbearing alone. This concept was initially suggested by our finding that bone changes in the proximal tibia and humerus of back-suspended rats were generally comparable (21). Since the hind limbs of these animals lost all weightbearing function whereas the forelimbs remained in contact with the floor of the model (Fig. 1), it was hypothesized that the skeletal abnormalities induced by back suspension were systemic rather than confined to the unloaded hind limbs. In view of the stressful situation to which back-suspended rats were subjected, as evidenced by lack of weight gain, hypersecretion of corticosteroids may be involved

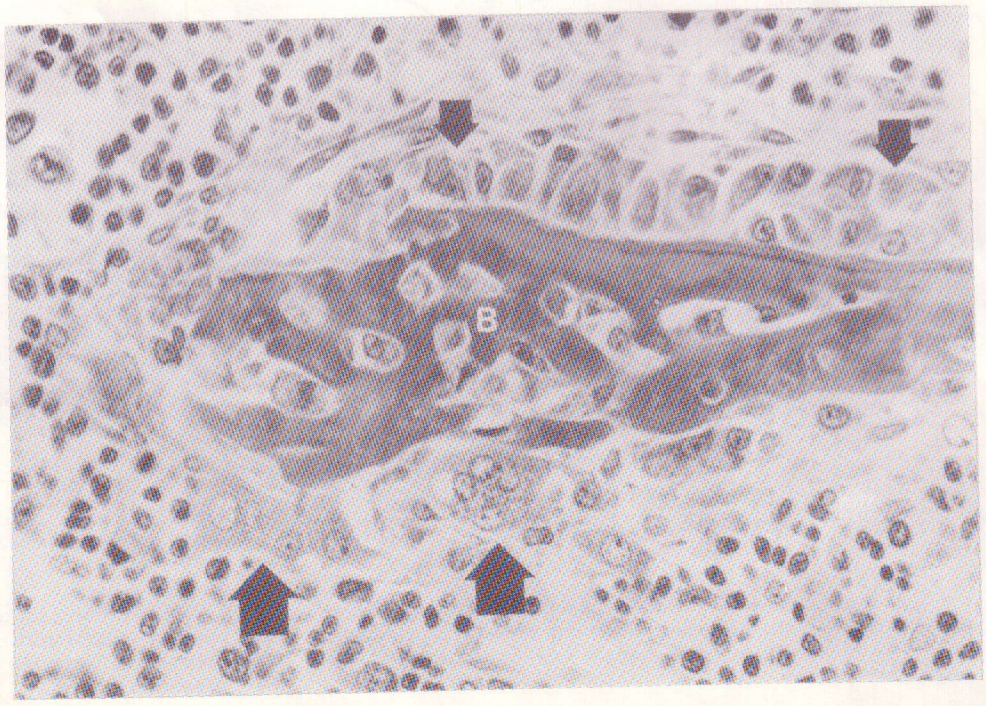

Fig. 5. A bone spicule (B) with adjacent osteoblasts (between small arrows) and osteoclasts (large arrows) in the proximal tibial metaphysis of a tail-suspended rat. X400.

in the etiology of these systemic skeletal defects. In a study designed to test this hypothesis, Feller *et al.* (4) determined that a transient, but significant, increase in plasma corticosterone and hypertrophy of adrenal tissue occurred in rats after 2 d of back suspension. However, subsequent measurements in back-suspended rats on days 3, 5, and 7 were similar to control values. Other investigators reported that adrenal hypertrophy persisted for the entire 1st week of hypokinesia in rats suspended from a harness (15). Nevertheless, an enhanced skeletal response to normal circulating levels of corticosterone could be explained on the basis of an increased density of glucocorticoid receptors at the target organ. Such a phenomenon has been described in atrophied leg muscles of suspended and immobilized rats (3,19).

The stresses associated with other forms of weightlessness and immobilization are thought to stimulate adrenal activity. For example, an acute threefold increase in urinary corticosteroid levels was reported in adult rhesus monkeys immobilized by chair restraint (10). Hypertrophied adrenal glands were detected in rats placed in orbit aboard Soviet Cosmos biosatellites (16). Skylab astronauts had elevated levels of plasma cortisol throughout the duration of their long-term missions (9). These findings suggest that the skeletal response to actual and simulated space flight may be mediated, in part, by corticosteroids.

Maintenance of normal weight is considered to be a general index of an animal's tolerance to experimental conditions. As mentioned above, lack of weight gain in back-suspended rats may be indicative of a stress response. This possibility, as well as the potential for inanition to affect the skeleton adversely (18), complicates interpretation of the results. In contrast, the tendency for tail-suspended rats to maintain normal rates of weight gain provides evidence for the less traumatic nature of this method of suspension. Therefore, tail suspension facilitates determination of the skeletal response to simulated weightlessness by minimizing undesirable side effects.

Longitudinal bone growth and the bone cell population in the proximal tibia of tail-suspended rats were relatively normal after 2 weeks of simulated weightlessness. Nevertheless, a decline in trabecular bone mass was evident in these animals. This finding agrees with a previous report that the tibiae of tail-suspended rats contain substantially less calcium than the tibiae of pair-fed control rats after 15 days of skeletal unloading (5). Bone loss in tail-suspended rats may be due to a transient inhibition of bone formation. Globus *et al.* (5) found that calcium uptake by the tibia was decreased in tail-suspended rats on the 5th day of simulated weightlessness but returned to control levels after the 10th day. These data suggest that an inhibition of bone formation occurs during the early stages of skeletal unloading. Histomorphometric findings of decreased osteoblast surface and number in the tibia on the 5th day of tail suspension support this concept (7). Therefore, trabecular bone loss despite normal histologic indices of bone resorption and formation on the 14th day of tail suspension could be accounted for by an earlier transient inhibition of bone formation.

In conclusion, the skeletal response of rats to simulated weightlessness for a 2-week period varied considerably according to the method of suspension. Back-suspended rats exhibited severe osteopenia and marked skeletal abnormalities in the proximal tibial metaphysis whereas the proximal tibia of tail-suspended rats, with the exception of mild osteopenia, was relatively normal by histologic criteria. These divergent results may be a consequence of a more pronounced stress response in back-suspended rats, as suggested by lack of weight gain in these animals. Maintenance of normal weight gain by tail-suspended rats provides evidence for the less traumatic nature of this method of suspension. Our findings indicate that tail suspension is preferable to back suspension for evaluating the effects of simulated weightlessness on skeletal homeostasis.

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